REMARKS

INTRODUCTORY COMMENTS:

Applicants acknowledge with appreciation the withdrawal of the rejection of claims 26 and 27 under 35 U.S.C. § 102(b) as being anticipated by Bernard.

STATUS OF THE CLAIMS:

Claims 26-37 are currently pending and under examination. By way of this amendment, claim 33 has been canceled without prejudice, and claims 30-32 have been amended.

Claims 26-37 were examined in the Office Action under reply, remaining claims 1-25 and 38-44 having been withdrawn from consideration as a result of restriction, which has now been made final. The examined claims stand rejected as follows:

(1) under 35 U.S.C. §102(b) as anticipated by Bernard et al. (claim 33);

(2) under 35 U.S.C. §103(a) as obvious over Bernard et al. in view of Chick et al. (claims 26-27, and 30-33);

(3) under 35 U.S.C. §103(a) as obvious over Bernard et al. in view of Chick et al., and further in view of Lee et al. (claims 28 and 29);

(4) under 35 U.S.C. §103(a) as obvious over Bernard et al. in view of Chick et al. and further in view of Dykens et al. (claims 34-37); and

(5) under 35 U.S.C. §112 as indefinite (claims 30-33).

The rejections are addressed in part by the cancellation of claim 33, and by amendment to claims 30-32, and are otherwise traversed for reasons that will be discussed in detail below.

Neither the cancellation of claims nor the amendment of pending claims should be construed as abandonment of any cancelled subject matter.

The amended claims are presented above in a "clean" form, as required under 37 C.F.R. § 1.121(c)(i). In addition, a marked up version of the amended claims, showing additions (underlined) and deletions (bracketed) are presented in the attached pages pursuant to 37 C.F.R § 1.121(c)(ii) indicated as Appendix A. A "clean" set of all the claims pending upon entry of the instant amendment is presented in Appendix B.

Applicants respectfully submit that these amendments do not introduce new matter. Applicants respectfully request entry and consideration of the claims as amended.

REJECTIONS UNDER 35 U.S.C. § 102(b)

The rejection of claims 26 and 27 under 35 U.S.C. § 102(b) as being anticipated by Bernard et al., (1998), *Anal. Biochem.* 255:101-107 ("Bernard") has been withdrawn. However, the Examiner has maintained the rejection of dependent claim 33 as being anticipated by Bernard.

Applicants hereby cancel claim 33 without prejudice to prosecution of the subject matter of claim 33 in a continuation application. Applicants therefore respectfully submit that the rejection of claim 33 under 35 U.S.C. § 102(b) is now moot, and ask that the rejection be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 112, second paragraph

Claims 30-33 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner alleged that claims 30-33 are broader than independent claim 26, from which they depend, because "claims 30-33 recite the broad recitation of the *binding pair* ***comprises*** *an enzyme-enzyme substrate pair (Claim 30), an anti-body-antigen pair (Claim 31), a biological receptor-ligand pair (Claim 32), and complementary oligonucleotides (Claim 33),*" while claim 26 recites that the "*binding pairs are selected from the group* ***consisting*** *of*... which is the narrower statement of the range/limitation". (Emphasis in original).

In response, claim 33 has been canceled, and claims 30-32 have been amended. The amended claims now recite that the binding pair ***is*** an enzyme-enyzme substrate pair, etc. Applicants therefore respectfully submit that the rejections have been overcome and request that the rejection of claims 30-33 under 35 U.S.C. § 112, second paragraph, be withdrawn.

THE 35 U.S.C. §103(a) REJECTION OF CLAIMS 28 AND 29 OVER BERNARD IN VIEW OF LEE

The rejection of claims 28 and 29 under 35 U.S.C. § 103(a) as obvious over Bernard in view of Lee has apparently been maintained, but not reiterated. According to the Examiner, Applicants' argument that the claimed invention is not concerned with DNA quenching of fluorescence is not convincing because "the fact that applicant has recognized another advantage that flows naturally from following the suggestion of the prior art cannot be the basis for patentability when the difference would otherwise be obvious."

Applicants respectfully traverse this rejection. Lee discussed the use of a linker only in the context of an oligonucleotide. The claims as amended do not encompass use with an

oligonucleotide. Further, as discussed in Applicants' previous response, the use of a linker as disclosed by Lee would not be suggested by the pending claims because Bernard specifically teaches that these two probes must be in close proximity in order for fluorescence energy transfer to occur. One skilled in the art would not read Lee in combination with Bernard and conclude that fluorescein and cyanine 5 would be efficient for fluorescence energy transfer if separated by even greater distances, such as would occur if the fluorophores were connected to the members of binding pairs by linkers, thereby separating the fluorophores further. Given the disclosures of Bernard and Lee, one skilled in the art would have no expectation of success, even if they considered attempting an experiment to determine if these two fluorophores could function when attached via linkers, out of concern for excessive separation between the fluorophores. In other words, Bernard teaches away from the use of linkers with fluorescein and cyanine 5.

Even if the Examiner is correct that one skilled in the art would be motivated to combine the teachings of Lee and Bernard and use fluorescein and cyanine 5 as an energy transfer pair when attached to a binding pair via a linker, Applicants submit that it would be merely obvious to try such an experiment, but not obvious that the experiment would be successful, especially when viewed in light of the long standing belief that substantial overlap between donor emission and acceptor excitation is necessary. Therefore, for this reason as well, the combination of the disclosures of Lee and Bernard does not render the present claims unpatentable.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 28 and 29 under 35 U.S.C. § 103(a) over Bernard in view of Lee.

THE 35 U.S.C. §103(a) REJECTION OF CLAIMS 30-32 OVER BERNARD IN VIEW OF CHICK

The rejection of claims 30-32 as obvious over Bernard in view of Chick appears to be maintained as well. The Examiner contends that preliminary work discussed in Bernard demonstrates that fluorescence energy transfer between fluorescein and cyanine 5 does occur, in contrast to Applicants' assertion that fluorescence energy transfer only occurs between cyanine 5 and fluorescein under very specific and restricted experimental conditions. The Examiner asserts that Bernard suggests that "some separation is more efficient than no separation, with gradual decrease in energy transfer efficiency as the separation increases…, which impl[ies] that distance would be a contributing factor in energy transfer between cyanine 5 and fluorescein." The Examiner also points to Bernard for additional discussion of resonance energy transfer designs that can be envisioned.

Applicants respectfully traverse this rejection. On inspection of the relevant section in Bernard, we see that instead of a configuration where the labels are placed on complementary strands, Bernard discusses the use of two adjacent oligonucleotide probes, each labeled with a single fluorophore. Bernard then cites Wittwer, et al., (1997), *Biotechniques* 22(1):130-138, a reference submitted in the Supplemental Information Disclosure Statement dated January 25, 2002. Applicants previously noted that Wittwer also relates to the use of fluorescein and cyanine 5 as dye pairs for fluorescence energy transfer for use in detecting DNA hybridization. In particular, Wittwer (and Bernard in the cited section) discusses the use of 5'-labeled-cyanine 5 and 3'-labeled- fluorescein probes, where the fluorophores are separated by one unpaired nucleotide, to detect product accumulation during PCR. Applicants reiterate that Wittwer describes the use of the fluorescein and cyanine 5 as a dye pair on adjacent hybridized probes in which the fluorophores were positioned only one base pair apart. (See Wittwer, pages 131 and 134.) The "preliminary work" of Bernard, cited by the Examiner, also relates to this use of cyanine-5 and fluorescein on adjacent hybridization probes. In this regard, Bernard observes that "a one base-pair separation is more efficient than no separation, with a gradual decrease in energy transfer efficiency as the number of separating nucleotides increases."

Therefore, Applicants respectfully disagree with the Examiner's view, and believe that Bernard actually teaches that resonance energy transfer only occurs when the fluorophores are oriented at the optimal orientation and distance, and only when the fluorophore separation is very close (reaching a maximum with a 3-4 base pair separation due to the helical geometry of the DNA helix, according to Bernard at page 106). In fact, Bernard's teaching that "one base pair separation is more efficient than no separation" is strong support for Applicants' assertion that Bernard teaches that fluorescence energy transfer only occurs between cyanine 5 and fluorescein when there is optimal orientation and distance between the fluorophores.

Accordingly, Applicants assert that claims 30-32 are patentable over the Bernard and Chick references and respectfully request withdrawal of this rejection under 35 U.S.C. §103(a).

THE 35 U.S.C. §103(a) REJECTION OF CLAIMS 34-37 OVER BERNARD IN VIEW OF DYKENS

The Examiner also appears to have maintained the rejection of claims 34-37 as obvious over Bernard in view of Dykens. The Examiner alleges that Applicants' arguments that Chick and Dykens would not use fluorescein with cyanine 5 because these fluorophores do not have overlapping excited stated energy levels is in conflict with statements concerning the factors that

control energy transfer efficiency. The Examiner alleges that for energy transfer to occur, there must be some overlap between the donor emission spectrum and the acceptor excitation spectrum.

Applicants respectfully traverse this rejection. Specifically, Chick teaches that "[a]ny appropriately selected donor-acceptor pair can be used, provided that the emission of the donor overlaps with the excitation spectra of the acceptor and both members can absorb light energy at one wavelength and emit light energy of a different wavelength." See col. 3, lines 9-13, col. 6, lines 19-21. Chick further states that "[t]he area of overlap between the donor emission and the acceptor absorbance spectra (which is the overlap integral) is of importance," referring to Figure 1 where the overlap integral between the donor emission spectrum and the acceptor excitation spectrum is graphically illustrated. Chick goes on to discuss the theory and efficiency of fluorescence energy transfer, including the importance of the overlap integral. See col. 8, lines 4-10, and col. 8, line 63- col. 9, line 8.

Applicants do not deny that for energy transfer to occur, there must be some overlap between the donor emission spectrum and the acceptor excitation spectrum. However, it is Applicants' position that the teachings of Chick would not lead one of skill in the art to use cyanine 5 and fluorescein as a fluorescence energy transfer pair because, according to Chick, only fluorophores with sufficiently overlapping excited state energy levels are attractive as fluorescence energy transfer dye pairs. In effect, Chick teaches away from the use of fluorescein with cyanine 5.

Thus, the teachings of Chick in combination with Bernard would not motivate one skilled in the art to use cyanine 5 and fluorescein as a fluorescence energy transfer pair because there is so little spectral overlap between these fluorophores. According to the teaching of Chick, one skilled in the art would have no reasonable expectation of success that the combination of fluorescein and cyanine 5 would exhibit fluorescence energy transfer at useful efficiencies. Even given the teaching of Bernard that these two fluorophores are capable of fluorescence energy transfer in the context of oligonucleotide hybridization, one skilled in the art would have no expectation of success in trying this dye pair in an experimental setting where the orientation and distance factors between the donor and acceptor fluorophores could not be predicted or controlled, such as antigen-antibody or enzyme-inhibitor contexts as claimed.

With respect to the rejection of the claims as obvious over Bernard in view of Dykens, Applicants respectfully submit that there would be no reasonable expectation of success in

achieving efficient energy transfer at the energy transfer distances disclosed in Dykens. The distances recited in Dykens merely encompass the possible ranges of energy transfer available within the constraints of physical reality, and by no means teach or suggest that fluorescein and cyanine 5 could participate in fluorescence energy transfer within those distances.

Dykens discusses the criteria for determining a suitable energy transfer pair, and teaches that the energy emission spectrum of the donor molecule should at least partially overlap the energy absorption spectrum of the acceptor. Dykens instructs one skilled in the art to choose energy transfer pairs based on a typical donor compound having an emission peak wavelength that is within several nm of the excitation peak wavelength of the acceptor compound, with a difference between the donor and acceptor peaks being typically from about 70 nm to about 20 nm or less. (See col. 23, lines 8-17.) In contrast, the difference between the donor and acceptor peaks of the claimed donor acceptor pair, fluorescein and cyanine 5, is 130 nm, since the emission peak for fluorescein is 519 nm, while the excitation peak for cyanine is 649 nm. (See Specification at page 3, second paragraph.) Thus, the teachings of Dykens teach away from the combination of fluorescein and cyanine 5 as an acceptable energy transfer dye pair.

Even if Dykens were construed to invite experimentation to determine if energy may be detectably transferred between a donor and acceptor pair having a larger difference between the emission and excitation peaks, Dykens does not teach or suggest the claimed fluorescein and cyanine 5 donor acceptor pair. Dykens even sets forth an extensive list of potential donor-acceptor pairs; however, none of the suggested combinations of donor acceptor pairs includes fluorescein and cyanine 5. Applicants thus respectfully submit that one skilled in the art would not be motivated to attempt energy transfer using cyanine 5 and fluorescein at the distances disclosed in Dykens.

Therefore, Applicants respectfully submit that the rejection of the claims as obvious under 35 U.S.C. §103(a) over Bernard in view of Chick or Dykens has been overcome, and respectfully request reconsideration and withdrawal of the rejection.

NEW REJECTIONS UNDER 35 U.S.C. § 103(a)

Claims 26, 27, and 30-33 again stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Bernard in view of Chick. The Examiner reiterates the grounds for rejection previously set forth, but now states that "Chick incorporated these different analytes (binding pair) because the binding ability of these different analytes can be detected in a wide range of

physiological concentrations." According to the Examiner, it would be obvious to combine the composition of Bernard with different combinations of binding pairs as taught by Chick, because the binding ability of these different analytes can be detected in a wide range of physiological concentrations.

Applicants respectfully traverse this rejection. The range of physiological concentrations of the different analytes is not an element of the pending claims. Even if the binding ability of the additional analytes does occur over a wide range of physiological concentrations, this would not provide motivation to one skilled in the art to attempt experimentation using cyanine 5 and fluorescein as a fluorescence energy transfer dye pair. Applicants maintain that there would be no reasonable expectation of success in attempting to adapt the teachings of Bernard to the additional analytes given the low spectral overlap between these two fluorophores.

Claims 28 and 29 stand rejected over Bernard, in view of Chick and further in view of Lee. The Examiner concedes that Bernard and Chick do not teach an indirect attachment of the fluorophores to the binding pair. However, the Examiner alleges that Lee discloses attachment of the fluorophores via a linker to the binding pair, and that the flexibility of the extrinsic probe when it is attached to a longer linker causes heterogeneity of distance which would provide a more efficient fluorescence energy transfer. The Examiner alleges that it would have been obvious to modify the fluorophores as taught by Lee.

Applicants respectfully traverse this rejection. Applicants submit that the presence of a linker would be expected to *increase* the distance between the fluorophores and binding pair, and therefore would likely *decrease* resonance energy transfer. In other words, there would be no expectation of success in making the modification, especially in view of the teaching of Dykens that at least partial overlap between donor emission spectrum and the acceptor excitation spectrum is needed, and that the difference between the donor emission peak and the acceptor excitation peak should be typically from about 70 nm to about 20 nm or less, as well as the teaching of Bernard and Wittwer regarding the optimal number of base pairs separating these fluorophores.

Therefore, Applicants respectfully submit that the combination of references does not render the claimed composition obvious under 35 U.S.C. §103(a), and respectfully request withdrawal of the rejection.

Claims 34-37 stand rejected over Bernard in view of Chick, and further in view of Dykens. The Examiner states that both Bernard and Chick differ from the claimed invention in not teaching the proximity of the fluorescence resonance energy transfer. However, the Examiner alleges that Dykens teaches that the efficiency of the resonance energy transfer is dictated by the proximity of the donor and acceptor, and it would have been obvious to modify the proximity distance as taught by Dykens for the advantage of providing a more efficient determination of the resonance energy transfer in different combinations of binding pairs. The Examiner also states that discovering the optimum or workable ranges, when the general conditions of a claim are disclosed in the prior art, involves only routine skill.

Applicants respectfully traverse this rejection. Given the teachings of Bernard that the resonance energy transfer for this dye pair only occurs within a narrow range of distances in the context of oligonucleotide hybridization, one skilled in the art would not be motivated to use cyanine 5 and fluorescein at the wide range of distances as claimed in claims 34-37. As discussed above, Dykens instructs one skilled in the art to choose energy transfer pairs based on a typical donor compound having an emission peak wavelength that is within several nm of the excitation peak wavelength of the acceptor compound, with a difference between the donor and acceptor peaks being typically from about 70 nm to about 20 nm or less. (See col. 23, lines 8-17.) In contrast, the difference between the donor and acceptor peaks of the claimed donor acceptor pair, fluorescein and cyanine 5, is <u>130 nm</u>, since the emission peak for fluorescein is 519 nm, while the excitation peak for cyanine 5 is 649 nm. (See Specification at page 3, second paragraph.) Thus, the disclosure of Dykens <u>teaches away</u> from the combination of fluorescein and cyanine 5 as an acceptable energy transfer dye pair. Therefore, Applicants submit that the combination of references does not render the claimed composition obvious, and request reconsideration and withdrawal of the new 35 U.S.C. § 103(a) rejections.

<u>OTHER REFERENCES NOTED</u>

The Examiner cited additional references in the Office Action dated October 25, 2001 to further show the state of the art with respect to fluorescence energy transfer in general, or to fluorescein and cyanine 5 dyes in general. The cited references include the following: U.S. Patent Nos. 6,037,130 to Tyagi; 6,232,130 to Wolf; 6,255,083 to Williams; 6,140,494 to Hamilton; 6,191,278 to Lee; 6,133,429 to Davis; 6,087,102 to Chenchik; 6,171,794 to Burchard; 6,287,768 to Chenchik; and WO 97/46714.

Applicants have reviewed these references and agree with the Examiner's implicit finding that the references do not disclose or suggest, either individually or in combination, the presently claimed invention.

In particular, Applicants believe that WO 97/46714 is particularly relevant to support Applicants' assertion that the claimed invention is patentable over all of the cited art. The first named inventor on this published patent application, Wittwer, is an author listed on the Bernard reference as well as numerous references cited by Bernard.

Applicants note that WO 97/46714 describes in detail the use of fluorescein and cyanine 5 as dye pairs for fluorescence energy transfer in the context of monitoring the formation of PCR amplification products. The Examiner's attention is directed to the following sections of this reference in particular: page 3, last paragraph –page 4, line 13; page 21, lines 26-34; page 27, lines 10-31; page 33, line 12-page 34, line 15; page 36, lines 1-13 and 25 through page 37, line 18; page 38, line 33 through page 39, line 9; page 40, lines 10-23; page 42, line 29 through page 43, line 4; page 45, line 35 through page 44, line13; page 63, lines 29-36; page 64, line 34 through page 65, line 2 and lines 13-20; and Figures 12 and 18.

WO 97/46714 focuses on the unique requirements imposed by using fluorescence energy transfer to monitor the formation of PCR products, due to problems caused by the high concentration of probe needed for hybridization monitoring. See page 34, lines 12-14. The reference describes the investigation of the ability of fluorescein and cyanine to function as a dye pair in the context of detection of PCR products, where the <u>fluorophores were placed at precise locations</u> on either the primer or probe oligonucleotides, and where the <u>exact distance between the fluorophores could be controlled</u>. See in particular Figures 12 and 18, where the dependence on the number of bases between the adjacent labeled oligonucleotides was investigated. The reference also reported that the best energy transfer between these fluorophores was seen with only 0 to 2 intervening nucleotides. See, *inter alia*, page 27, line 26. The reference also discusses the effect of the position on the helix and states that "[h]aving the resonance energy transfer pair on adjacent nucleotides is not necessarily beneficial because the distance between the resonance energy transfer pair is effected by the position on the DNA helix." See page 63, lines 34-36. Thus, WO 97/46714 describes in fair detail the difficulties that must be solved in monitoring the generation of PCR products, and emphasizes the need for precise placement of fluorophores in order for experiments using fluorescein and cyanine 5 to be successful.

Applicants point out that the very same factors which enable cyanine 5 and fluorescein to function as probes of PCR product accumulation- namely avoiding the high background fluorescence (due to the high concentrations of probe required) and the ability to predictably place the fluorophores within fluorescence energy transfer proximity of one another (such as at the termini of adjacent oligonucleotide probes)- teach away from the use of cyanine 5 and fluorescein in situations where background fluorescence is not an issue and where the distance between the fluorophores is not readily under the experimenter's control.

WO 97/46714 also teaches away from applications of fluorescein and cyanine 5 as a fluorescence energy transfer pair outside the realm of oligonucleotide hybridization applications because it reinforces the prevailing belief in the art that "[i]ntuitively, the fluorescein emission and cy5 absorption do not overlap enough for resonance energy transfer to be considered." (See page 37, lines 7-8.)

Therefore, Applicants respectfully submit that the teachings of the art at the time the invention was made overwhelmingly taught against the use of fluorescein and cyanine 5 as a fluroscecence energy transfer dye pair useful in the context of antigen-antibody, biotin-avidin, biotin-streptavidin, hormone-hormone binding protein, receptor-ligand, IgG-protein A, lectin-carbohydrate, enzyme-enzyme cofactor, enzyme-enzyme inhibitor and enzyme-substrate interactions, as claimed in independent claim 26. One skilled in the art would have had no expectation of success in using the claimed dye pair outside the context of oligonucleotide hybridization experiments.

CONCLUSION

In sum, it is submitted that the claims, as amended, are patentable over the art. Accordingly, the application is now in condition for allowance. A Notice of Allowance is requested, and a prompt mailing thereof would be much appreciated.

If the Examiner has any questions or wishes to discuss the matter further, he may contact the undersigned attorney at (650) 330-4917.

Respectfully submitted,

By: ____________________
Cynthia R. Moore
Registration No. 46,086

REED & ASSOCIATES
800 Menlo Avenue, Suite 210
Menlo Park, California 94025
(650) 330-0900 Telephone
(650) 330-0980 Facsimile

APPENDIX A

MARKED UP VERSION OF THE CLAIMS UNDER 37 C.F.R. § 1.121(c)(ii)

30. (Amended)

The composition of claim 26, wherein the binding pair is ~~comprises~~ an enzyme-enzyme substrate pair.

31. (Amended)

The composition of claim 26, wherein the binding pair is ~~comprises~~ an antibody-antigen pair.

32. (Amended)

The composition of claim 26, wherein the binding pair is ~~comprises~~ a biological receptor-ligand pair.

APPENDIX B

CLAIMS PENDING UPON ENTRY OF THIS AMENDMENT

26. (Amended) A composition comprising a first member of a binding pair directly or indirectly attached to fluorescein and a second member of the binding pair directly or indirectly attached to cyanine 5, wherein the first and second members of the binding pair are associated so that the fluorescein and cyanine 5 are in fluorescence resonance energy transfer proximity to each other, wherein said binding pairs are selected from the group consisting of antigen-antibody, biotin-avidin, biotin-streptavidin, hormone-hormone binding protein, receptor-ligand, IgG-protein A, lectin-carbohydrate, enzyme-enzyme cofactor, enzyme-enzyme inhibitor, enzyme-substrate and fragments or analogs thereof.

27. The composition of claim 26, wherein the first member of the binding pair is directly attached to fluorescein and the second member of the binding pair is directly attached to cyanine 5, and the direct attachment is effected through a covalent bond.

28. The composition of claim 26, wherein the first member of the binding pair is indirectly attached to fluorescein and the second member of the binding pair is indirectly attached to cyanine 5, and the indirect attachment is effected through one or more linking moieties.

29. The composition of claim 26, wherein at least one member of the binding pair is indirectly attached to either fluorescein or cyanine 5, and the indirect attachment is effected through one or more linking moieties.

30. (Amended) The composition of claim 26, wherein the binding pair is an enzyme-enzyme substrate pair.

31. (Amended) The composition of claim 26, wherein the binding pair is an antibody-antigen pair.

32. (Amended) The composition of claim 26, wherein the binding pair is a biological receptor-ligand pair.

34. The composition of claim 26, wherein the fluorescence resonance energy transfer proximity is about 1 Å to about 100 Å.

35. The composition of claim 34, wherein the fluorescence resonance energy transfer proximity is between about 5 Å to about 80 Å.

36. The composition of claim 35, wherein the fluorescence resonance energy transfer proximity is between about 10 Å to about 70 Å.

37. The composition of claim 36, wherein the fluorescence resonance energy transfer proximity is between about 20 Å to about 60 Å.